The effectiveness of aquatic plants as surrogates for wider biodiversity in standing fresh waters

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Summary

1. Fresh waters are among the most globally threatened habitats and their biodiversity is declining at an unparalleled rate. In an attempt to slow this decline, multiple approaches have been used to conserve, restore or enhance waterbodies. However, evaluating their effectiveness is time-consuming and expensive. Identifying species or assemblages across a range of ecological conditions that can provide a surrogate for wider freshwater biodiversity is therefore of significant value for conservation management and planning.
2. For lakes and ponds in three contrasting landscapes of Britain (lowland agricultural, eastern England; upland, north-west England; urban, central Scotland) we examined the link between macrophyte species, macrophyte morpho-group diversity (an indicator of structural diversity) and the richness of three widespread aquatic macroinvertebrate groups (molluscs, beetles and odonates) using structural equation modelling. We hypothesised that increased macrophyte richness and, hence, increased vegetation structural complexity, would increase macroinvertebrate richness after accounting for local and landscape conditions.
3. We found that macrophyte richness, via macrophyte morpho-group diversity, were an effective surrogate for mollusc, beetle and odonate richness in ponds after accounting for variation caused by physical variables, water chemistry and surrounding land use. However, only mollusc richness could be predicted by macrophyte morpho-group diversity in lakes, with no significant predicted effect on beetles or odonates.
4. Our results indicate that macrophyte morpho-group diversity can be viewed as a suitable surrogate of macroinvertebrate biodiversity across diverse landscapes, particularly in ponds and to a lesser extent in lakes. This has important implications for the restoration, conservation and creation of standing water habitats and for

assessing their effectiveness in addressing the decline of global freshwater biodiversity. Management actions prioritising the development of species-rich and structurally diverse macrophyte assemblages will likely benefit wider freshwater biodiversity.

Introduction

A biological surrogate, indicator or proxy is an individual or group of organisms that can be used to identify a healthy, biodiverse or functional ecosystem, or to infer environmental conditions existing now or in the past. Such surrogates are commonly used in conservation decision making and offer a means of choosing and tracking the effectiveness of management approaches, with the premise that, if the surrogate is protected and conserved, there will be wider biodiversity and ecosystem benefits (Caro, 2010). A further advantage of surrogates is reduced reliance on large-scale, multi-taxon surveys which are time-consuming, expensive and often require specialist knowledge. Quantifying the link between surrogates and wider biodiversity or functioning of an ecosystem is crucial for validation, yet numerous studies conducted across several ecosystems and species have failed to identify consistent, reliable surrogates of either biodiversity, ecosystem function or phylogenetic diversity (Heino, 2015; Rapacciuolo *et al.*, 2018). Despite this, improved ecological knowledge and data accessibility, alongside advancing analytical tools, offer renewed promise in the search for surrogates. This is particularly relevant in freshwater ecosystems as they are one of the most globally threatened habitats due to the scale of humans impacts (Reid *et al.*, 2018; WWF, 2018).

Numerous studies have sought to evaluate surrogacy in freshwaters, with macroinvertebrates receiving most attention. For ponds and rivers there is broad consensus that a few species-rich invertebrate groups (e.g. Coleoptera, Odonata, Mollusca and Trichoptera) are broadly representative of wider macroinvertebrate assemblages (Briers & Biggs, 2003; Bilton *et al.*, 2006; Sánchez-Fernández *et al.*, 2006; Ruhí & Batzer, 2014; Guan *et al.*, 2018). However, where surrogacy across different taxonomic groups has been studied e.g. plants or amphibians to macroinvertebrates, the results have been inconsistent, with relationships variously non-existent (Santi *et al.*, 2010; Guareschi *et al.*, 2015), weak (Heino,

2010; Kirkman *et al.*, 2012; Rooney & Bayley, 2012; Ilg & Oertli, 2017), moderate (Santi *et al.*, 2010; Gioria *et al.*, 2010) or strong (Janssen *et al.*, 2018). Most previous research has concentrated on one or two taxonomic groups, focussing on a single habitat type, distributed over a small geographical range. Therefore, even at small-scales, there is limited evidence of effective surrogates for wider freshwater biodiversity.

Aquatic plants (macrophytes), encompassing bryophytes, macroalgae and vascular plants, are a fundamental component of aquatic food webs and play a central role in nutrient flux within freshwater habitats, linking atmosphere, soil and water. They influence the quality of the surrounding aquatic environment by creating structurally-complex habitats comprised of submerged, floating and emergent vegetation, where differences in leaf and stem architecture (e.g. floating vs. simple linear vs. dendritic leaves) between species, diversifies habitat complexity where it might otherwise be low (Jeppesen *et al.*, 1998). Furthermore, as primary producers, macrophytes influence water chemistry, provide food for grazers, habitats for egg-laying, whilst also mediating predator-prey interactions through provision of refugia for prey and concealment for predators (Diehl & Kornijow, 1998; Jeppesen *et al.*, 1998). A shared response to environmental conditions is often believed to be a key driver of species surrogacy (Gioria, Bacaro & Feehan, 2011; Rooney & Bayley, 2012), but, given the key structuring role of macrophytes, and their potential to operate as ecosystem engineers (Gurnell *et al.*, 2013), it seems highly likely that their presence and richness will directly or indirectly govern the availability of resources to, and environmental suitability for, other species. Since they are taxonomically and ecologically well understood and occur in almost all freshwater habitat types globally, macrophytes may thus be an ideal surrogate for wider freshwater biodiversity.

To our knowledge, the influence of macrophyte richness on multiple aquatic biota, across different freshwater habitats and covering environmentally diverse conditions has not

103 previously been examined. Therefore, current understanding of the potential value of
104 macrophytes as a surrogate is constrained. In this study, aquatic molluscs, aquatic beetles and
105 odonates were selected as focal biota due to their high taxonomic diversity, widespread
106 distribution in standing fresh waters and because all three groups include species of
107 conservation concern. Our primary objective was to test whether macrophytes act as
108 surrogates for wider freshwater biodiversity across three contrasting (agricultural, upland and
109 urban), but typical aquatic landscapes (so-called 'hydroscapes'). We did this by assessing the
110 strength of chemical and physical drivers and surrounding land use in explaining waterbody-
111 scale richness of the biota. At the same time, we additionally tested if macrophyte species
112 richness, mediated through morpho-group diversity, could further explain macroinvertebrate
113 richness. We hypothesised that waterbodies with higher macrophyte richness, and, hence,
114 greater macrophyte morpho-group diversity (an indicator of structural diversity), would have
115 greater macroinvertebrate richness, with the former being a stronger predictor than chemical,
116 physical and surrounding land use. However, further macroinvertebrate assemblage-specific
117 effects are expected, reflecting either differences in the degree of dependence on macrophytes
118 for habitat support, or habitat type-specific (pond or lake) differences in the importance of
119 macrophytes as a component of habitat diversity.
120
121 **Methods**
122 *Study areas and data collection*
123 Three contrasting landscapes were chosen within Britain to account for different
124 combinations of stressors associated with different land use types; lowland agricultural
125 (north-east Norfolk, eastern England), upland (Cumbria, north-west England) and urban
126 (Greater Glasgow, central Scotland). Within each of these hydroscapes, 22-29 replicates of
127 both lakes and ponds were sampled. In this study, lakes were defined as waterbodies with

surface area > 1 ha, while ponds were < 1 ha in area and generally shallow (< 2 m max. depth). Both categories included man-made and natural waterbodies. Within each of these waterbodies four taxonomic groups were selected to cover a range of habitat requirements, pollutant sensitivities and dispersal abilities, namely macrophytes (as surrogates), aquatic molluscs, aquatic beetles (hereafter referred to as molluscs and beetles) and odonates (dragon and damselflies). Extensive data on these taxonomic groups were obtained via national recorders (i.e. Aquatic Coleoptera Conservation Trust, British Conchological Society and British Dragonfly Society), while water chemistry, where available, and data on macrophytes from commissioned surveys, was provided by UK environmental agencies or the Joint Nature Conservation Committee (JNCC). All data were closely scrutinised to ensure inter-compatibility, with multi-visit, full inventory surveys prioritised. Only records from the last decade were retained. The availability of multiple recent records of adult odonates influenced site selection because favourable weather conditions for surveying these could not be guaranteed during field campaigns conducted for this study. Where gaps in the data existed or when a greater number of replicate waterbodies were needed, new data were collected during June to August of 2016-17. Several sites had data collected for all species assemblages and 88% of the sites used in the study were visited by the authors to gather additional data for at least one species assemblage or to collect water samples for water chemistry analysis (Table S1).

For each waterbody, the following physical variables were derived from the UK Lakes Portal (https://eip.ceh.ac.uk/apps/lakes/index.html); altitude, area, catchment size, perimeter, ratio of waterbody to catchment area and shoreline development index (indicating shape complexity of the shoreline). For water chemistry data provided by UK environmental agencies, a mean value was taken for each variable based on samples collected in summer (June-September). In all other cases we collected a water sample from the middle of each site

and measured conductivity, dissolved oxygen, oxygen saturation, pH and temperature in the field using a HACH HQ30d meter. Alkalinity was also measured in the field by titration using sulphuric acid with a HACH AL-DT kit. A 500 ml subsample was filtered (47 mm glass microfiber, 1.2 μm pore Whatman GF/C filters) within 12 hours of collection and analysed for major nutrients and metals (see Table S2 for a list of determinands). Chlorophyll *a* was determined by extraction by soaking filters in 90% methanol overnight and quantification by spectrophotometry.

For surveys of biota, exhaustive inventory sampling was conducted for each taxon group covering the complete margin of each waterbody. Macrophytes were recorded from the marginal zone to the maximum growing depth, assisted by use of a double-headed rake and/or a bathyscope for deeper water or where visibility was poor. For ponds, the entire water area was surveyed. For lakes, three or four sectors, each covering 100 m of shoreline, were surveyed to account for variation in exposure, shading, water depth and littoral substrate, following the JNCC survey methodology (Interagency Freshwater Group 2015). Within each sector, five transects were established perpendicular to the shore and four replicate quadrats were sampled per transect at depths of 0.25 m, 0.50 m, 0.75 m and >0.75 m, respectively, giving a total of 60 to 80 quadrats per lake. A boat was used to survey areas that were too deep for survey by wading (>75 cm).

Molluscs, beetles and larval odonates were sampled using a 1 mm mesh pond net. For each waterbody, the number of mesohabitats (e.g. rocky substrate, floating leaved, short/tall emergent, or submerged vegetation) was visually assessed and all were then sampled by sweeping the pond net through the water column and any vegetation present. This was repeated in each mesohabitat until no more new species could easily be found. The sample was live sorted and individuals were identified to species level in the field and released. When individuals could not be identified in the field they were preserved in 70% industrial

methylated spirits (IMS) and identified to species-level, wherever possible. Adult odonates were identified visually in the field, assisted by use of binoculars. Where individuals within a taxonomic group were identified to mixed resolution, only the highest resolution records were used.

Land cover and connectivity

Land Cover Maps (Rowland *et al.*, 2017) were used to assess land use within the upstream catchment of each waterbody (representing hydrological connectivity), and within buffers of 50 m, 100 m, 500 m and 1 km surrounding each waterbody (representing riparian and aerial connectivity). To reduce the number of interrelated land cover categories, a series of composites were created; agricultural (arable and horticulture + improved grassland); urban (suburban + urban) and wetland (fen, marsh and swamp + bog). Within each waterbody buffer or catchment, land cover classes were expressed as a percentage of the total buffer or catchment area (minus the area occupied by the focal waterbody). Since freshwater and wetland land cover classes exhibited a high number of zero or low values these classes were transformed to absence (-1) and presence (1) to make their effect sizes directly comparable with those of continuous predictors.

Variable selection and statistical analyses

Species richness was defined as the number of macrophyte or macroinvertebrate species per waterbody (or highest taxonomic resolution). Macrophyte morpho-group diversity was derived by assigning each species to one of 26 morpho-groups based on a library of morphological and regenerative traits (Willby, Abernethy & Demars, 2000), but expanded to incorporate bryophytes, macroalgae and a wide range of emergent species (Table S3). To determine if a sufficient number of waterbodies were surveyed per hydroscape for the

four taxonomic groups, sample coverage was calculated based on incidence data per waterbody using the iNEXT library (Hsieh, Ma & Chao, 2016). Prior to statistical analyses all continuous explanatory variables (excluding pH) were log transformed, mean centred and scaled by 1 SD, to improve comparability between variables and to reduce the effect of outliers (full set of continuous variables given in Figure S1). 64% of ponds sampled (especially those <0.1 ha) did not have definable catchments, so a binary 'catchment present' category was created for all ponds. Binary explanatory variables (e.g. catchment present for ponds, outflow and inflow) were transformed to have values of –1 (absent) and 1 (present).

To reduce model complexity principal components analysis (PCA) was applied to separate sets of water chemistry, physical and land use variables to identify those variables that maximised variation amongst sites (Figure S1). All continuous explanatory variables (excluding pH) were log transformed, mean centred and scaled by 1 SD, to improve comparability between variables and to reduce the effect of outliers. Correlations between predictor variables were then assessed in a correlation matrix (Figure S1) and checked for variance inflation (VIF). Where variables were highly correlative (VIF > 20) they were removed. The remaining variables were then used as explanatory variables for macrophyte species richness in a linear model (LM) with model-averaging then implemented (Burnham & Anderson, 2002). Variables that significantly explained macrophyte richness, based on the sums of Akaike weights (Figure S1), were then retained.

A conceptual model was developed to incorporate expected relationships between species richness and explanatory variables (Fig. 1). This model was based on the simple hypothesis that connectivity, land use and waterbody physical and water chemistry variables influence macrophyte species richness to a greater extent than macrophyte morpho-group diversity or richness of the macroinvertebrate groups, and that it is predominantly via macrophytes that these environmental effects are transmitted to macroinvertebrates. We also

hypothesised that macrophyte morpho-group diversity would be a more important determinant of macroinvertebrate richness than macrophyte taxonomic richness due to the increased structural complexity that a high richness of macrophyte morpho-groups provides. We used structural equation modelling (SEM) to quantify the direct and indirect effects of these explanatory variables on macrophyte richness, macrophyte morpho-group diversity and macroinvertebrate richness. SEMs are a multivariate technique based on constituent LMs that allow standardised comparisons of direct and indirect relationships. Constituent LMs were created and residuals assessed to determine if they met linear model assumptions and examined for spatial autocorrelation using Moran's I statistic. All constituent LMs met linear model assumptions and no significant patterns in spatial autocorrelation were detected ($P > 0.05$). Bivariate relationships between each response and explanatory variable were explored graphically to identify potential non-linear relationships. Where non-linear relationships were found, the explanatory variable was converted to second degree orthogonal polynomials. No multicollinearity was detected in constituent LMs with a VIF threshold of < 5. During SEM model evaluation, missing pathways (i.e. previously unconsidered significant relationships) were identified and incorporated into the final SEM. Model fit was assessed using Fisher's C, where values of $P > 0.05$ indicated that the model was supported by the observed data. The term hydroscape ('Agricultural', 'Upland' and 'Urban') was added to each constituent LM, but was never significant and often increased the VIF due to correlations with land use. Hydroscape was then added as a random effect to each constituent LM, but did not improve the AIC. Therefore, the term hydroscape was not included in the final SEMs.

All statistical analysis was conducted using RStudio (R Core Team, 2018) with the libraries: piecewiseSEM (Lefcheck, 2016), sp (Bivand, Pebesma & Gomez-Rubio, 2013), sjPlot (Lüdecke, 2018), MuMIn (Bartoń, 2018), ggbiplot (Vu, 2011), factoextra (Kassambara

252 & Mundt, 2017), FactoMineR (Le, Josse & Husson, 2008), iNEXT (Hsieh *et al.*, 2016) and
253 spdep (Bivand, Hauke & Kossowski, 2015).
254

255 **Results**

256 The *a priori* designation of the three hydroscapes as upland, urban or agricultural was
257 confirmed by analysis of the catchment characteristics of their constituent waterbodies (Table
258 1).

259 In total 176, 52, 249 and 35 species of macrophyte, mollusc, beetle and odonates
260 respectively were recorded across the 158 waterbodies, studied via a combination of our
261 surveys and archived data. Estimated sample coverage was generally high (mean = 94%)
262 indicating effective sampling of each taxonomic group per waterbody type per hydroscape
263 (Table 2). Further details of the sampling efficiency and completeness can be found in Figure
264 S2 in the supporting information.

265 For both lakes and ponds, correlations in raw species richness was compared amongst
266 the taxonomic groups (Figure S3), but none were found to be significant. Therefore,
267 environment variables have to be considered in order to deduce true correlative relationships
268 between the taxonomic groups.

269 Our conceptual model (Fig. 1) was poorly supported for both lakes and ponds, with
270 multiple missing significant pathways being identified. However, with the addition of these
271 pathways to the SEM (Table S4) the goodness-of-fit for both models reproduced the data
272 well (lakes: Fisher's C = 162.3, df = 164, P = 0.523; ponds: Fisher's C = 121.2, df = 124, P =
273 0.554). Unstandardised and standardised effect sizes of all explanatory variables for lakes and
274 ponds are provided in Table S5.

275 In lakes, macrophyte richness was explained principally by water chemistry and to a
276 lesser extent by nearby land use (R^2 = 0.64) (Fig. 2). Variables indicative of nutrient-
277 enrichment or poor water quality (nitrate, total phosphorus and water colour) negatively
278 affected macrophyte richness, with nearby agricultural land positively influencing
279 macrophyte morpho-group diversity was, as expected, strongly related

to macrophyte richness. However, the subsequent effect on macroinvertebrates was varied; macrophyte morpho-group diversity positively influenced mollusc richness, but had no effect on beetle and odonate richness. For the latter groups, environmental conditions (i.e. land use and waterbody physical variables) were more influential. Increasing altitude was a strong, negative determinant of both mollusc and odonate richness, with reasonable variance explained for both assemblages (R^2 = 0.76 and 0.36). The explained variance in beetle richness was the lowest of all the taxonomic groups (R^2 = 0.29) with only wetlands in the catchment and nearby agricultural land positively affecting richness and, to a lesser extent, lakes with relatively large catchments having a negative effect.

For ponds, nearby surrounding land use had no significant impact on macrophyte richness compared to the influence of water chemistry (principally conductivity and pH) and presence of an outflow (Fig. 3). Macrophyte morpho-group diversity was again strongly related to macrophyte richness, whilst ammonium and nearby urban land use also had minor negative effects on morpho-group diversity. The degree of urbanisation within 500 m of a pond had contrasting effects on macroinvertebrate biota, being positive for molluscs, but highly negative for beetles and odonates. A negative effect of altitude was observed for mollusc and beetle richness in ponds, as with lakes. Nevertheless, despite some variation being explained by physical variables, water chemistry and land use, an increased macrophyte morpho-group diversity had a significant positive effect on all macroinvertebrate groups.

Discussion

Simple surrogates for freshwater biodiversity should help to inform choices over the protection, restoration or creation of waterbodies, and in monitoring the effectiveness of related actions. However, few studies have sought out a surrogate appropriate for multiple freshwater habitats and disparate species assemblages over large spatial scales. We found that, regardless of the landscape, high macrophyte richness, specifically via high morpho-group diversity, was a suitable surrogate for a higher richness of multiple macroinvertebrate species assemblages (molluscs, beetles and odonates) in ponds, but only mollusc richness could be predicted by macrophyte morpho-group diversity in lakes.

The drivers of species richness

Land use is often assumed to be a major driver of species composition as it provides a proxy for stressors (e.g. agriculturally-derived nutrients or pollutants originating from urban areas) (Hassall, 2014) or affects spatial processes (altering connectivity both positively and negatively) (Hill *et al.*, 2017). Urbanisation is assumed to be indicative of reduced connectivity due to the density of roads and built-up areas that restrict dispersal between waterbodies (Hassall, 2014). Moreover, previous studies of ponds and rivers indicate that active dispersers were less restricted by habitat structure than passive dispersers (Hill *et al.*, 2017; Sarremejane *et al.*, 2017). In our study, urban land use had a negative effect on actively-dispersing odonates and beetles in ponds, suggesting that an active dispersal ability may be insufficient to counteract effects of urbanisation and the associated changes to local habitat structure that urbanisation produces. However, urban land use was positively associated with passively dispersing molluscs. This latter finding may reflect the increased presence of vectors within the local landscape (for example waterfowl attracted by supplementary feeding may increase bird-mediated dispersal (van Leeuwen *et al.*, 2012;

Simonová *et al.*, 2016)), combined with molluscs' tolerance of productive poorly oxygenated conditions. Alternatively, the increased concentrations of some major ions due to rural and urban run-off may also benefit molluscs since calcium is used for shell construction (Moss, 2017). It was expected that adjacent agricultural land use would negatively affect biodiversity due to increased nutrient or fine sediment inputs, yet agriculture within 500 m of lakes had a slight positive effect on lake macrophyte richness. However, the interpretation that agriculture is positive for biodiversity should be taken with caution, since in the composite LMs that underpin the SEM, agricultural land use in the catchment as a whole had a non-linear relationship with macrophyte richness, becoming negative when agricultural extent exceeded ~40% (though this was not significant in the final model). Freshwaters and wetlands in the catchment or buffers were expected to positively affect biodiversity as they potentially increase connectivity, and therefore resilience, by acting as stepping stones (Biggs *et al.*, 2005). Although we observed a positive effect of nearby wetlands (within a 500 m buffer), or wetlands in the catchment on lake beetles and molluscs, respectively, this was secondary to waterbody-specific influences (e.g. altitude and water chemistry), consistent with other studies (Hill *et al.*, 2017; Thornhill *et al.*, 2017). Water chemistry influenced macrophyte richness in both lakes and ponds, with variables indicative of nutrient-enrichment negatively affecting richness. Alkalinity had a negative effect on lake macrophytes, which was unexpected as previous work has generally shown a positive influence of alkalinity on macrophyte richness (Vestergaard & Sand-Jensen, 2000). The effect we observed was most likely driven by a strong correlation between alkalinity and total oxidised nitrogen or conductivity (Figure S1), indicative of declining water quality (Heegaard *et al.*, 2001). Waterbody chemistry had few direct effects on the studied macroinvertebrate groups and it is therefore likely that macrophytes mediate nutrient-enrichment effects (Declerck *et al.*, 2005).

Identifying a simple surrogate of diverse and complex species assemblages that transcends multiple, potentially interacting variables which vary both temporally and spatially is difficult, with few variables seemingly transferable across habitat types, regions and species assemblages (Batzer, 2013). Macrophyte richness and composition have previously been shown to positively affect macroinvertebrate assemblages in multiple freshwater habitats; ponds (Palmer, 1981; Gioria *et al.*, 2011), wetlands (Kirkman *et al.*, 2012), lakes (Heino & Tolonen, 2017) and rivers (Holmes & Raven, 2014). However, the drivers of species surrogacy are mostly speculative rather than explicitly studied. In our study, the most plausible basis for the surrogacy we observed is that good water quality allows for high macrophyte richness, which leads to a greater diversity of macrophyte morpho-groups and macroinvertebrate richness benefits through provision of increased architectural complexity. These benefits are probably group- or life stage-specific. For example, molluscs may benefit from high macrophyte richness due to increased food resources, reduced predation and increased microhabitat diversity (Brönmark, 1985). Beetles may benefit from the heterogenous substrate available for egg-laying, refugia and through increased prey availability (Bloechl *et al.*, 2010). Furthermore, adult odonates use emergent macrophytes for perching, egg-laying and emergence (Le Gall *et al.*, 2018), whereas their larvae use submerged macrophytes for shelter and foraging (Goertzen & Suhling, 2013). A greater macrophyte morpho-group richness linked to asynchronous growth peaks may also extend the duration of macrophyte cover (van Donk & Gulati, 1995; Sayer, Davidson & Jones, 2010) which should benefit macroinvertebrates, but this area is relatively unexplored.

It is also possible that some macroinvertebrate groups may influence the richness of others, for example, via predation. However, as positive or negative pathways between any of the macroinvertebrate groups were not identified in our analysis, we can hypothesize that the effect of predation on richness are low, relative to the effect of macrophytes. Differences in

376 explained variance amongst macroinvertebrates were reasonably consistent across waterbody
377 types, with mollusc richness highest followed by odonates and then beetles. The low
378 explained variance observed for beetles may in part reflect the high species richness found.
379 Beetles are one of the most speciose groups globally with a wide geographical and ecological
380 range (Bilton *et al.*, 2006); moreover, the balance between habitat specialists and generalists
381 will be masked when considering diversity only in terms of species richness.
382 The strength of the surrogacy between macrophytes and macroinvertebrates differed
383 between waterbody types, with macrophyte richness being a stronger driver of
384 macroinvertebrate richness in ponds than lakes. This pattern may arise because lakes are
385 more likely to support large populations of fish, which are known to exert strong predation
386 pressure on macroinvertebrates (Diehl, 1992; Jones & Sayer, 2003). Molluscs, for example,
387 are commonly consumed by fish with resulting reductions in density, although effects on
388 richness are less understood (Dillon, 2000). Fish could also influence macroinvertebrates
389 indirectly via various cascading effects on macrophyte diversity caused by herbivory
390 (Matsuzaki *et al.*, 2009), zooplanktivory (Jeppesen *et al.*, 1998) or benthivory, particularly in
391 shallow lakes (Kloskowski, 2011). Both abundance of macrophytes and macroinvertebrates
392 will also be affected by waterfowl herbivory and bioturbation (Rodríguez-Pérez & Green,
393 2012; Wood *et al.*, 2012), with lakes likely to support greater waterfowl densities than ponds.
394 A further factor affecting macroinvertebrate diversity in lakes may be physical disturbance of
395 the shoreline due to wave action, which is much more intense in lakes than ponds due to an
396 increased fetch (Fairchild, Faulds & Matta, 2000). Given that our focal macroinvertebrate
397 groups, molluscs in particular, are poorly stream-lined and prone to being dislodged by
398 currents, their link with macrophyte diversity may reflect a shared need for sheltered
399 marginal habitats. In this study, it is likely that the effects of fish predation or physical
400 disturbance on macroinvertebrate richness is mediated through macrophyte morpho-group

401 diversity, as found in Cladocera (Burks, Jeppesen & Lodge, 2007), but further study would
402 be useful to tease apart the multiple interacting processes involved (see Dillon (2000) for a
403 review). Moreover, future studies should endeavour to determine fish abundance. As fish can
404 be important drivers of aquatic community composition (Scheffer *et al.*, 2006), their
405 inclusion will undoubtedly improve the predictive power of models and therefore the
406 application of surrogates in other freshwater habitats.
407
408 *Surrogacy and available statistical tools*
409 The search for widely applicable and robust surrogates of freshwater biodiversity has
410 probably been somewhat confounded by the differing statistical approaches used to detect
411 surrogacy (Gioria *et al.*, 2011). The majority of studies have tested congruence between
412 species assemblages by using multivariate ordination to consider the influence of local
413 environmental variables (Declerck *et al.*, 2005; Bilton *et al.*, 2006; Santi *et al.*, 2010; Gioria
414 *et al.*, 2010; Guareschi *et al.*, 2015). Others have utilised Mantel tests (Heino, 2010; Rooney
415 & Bayley, 2012; Ruhí & Batzer, 2014; Ilg & Oertli, 2017), species correlations (Sánchez-
416 Fernández *et al.*, 2006; Slimani *et al.*, 2019) or a Species Accumulation Index (Kirkman *et*
417 *al.*, 2012). In addition to the range of analytical methods used, the choice of diversity index
418 for assessing surrogacy also influences outcomes, with alternative measures of alpha
419 diversity (e.g. richness, functional and phylogenetic alpha) varying in their sensitivity to
420 environmental drivers (Heino & Tolonen, 2017). To our knowledge SEMs have not been
421 previously utilised in the quest for surrogacy in freshwater ecology. The advantage of SEMs
422 is that disparate species assemblages can be analysed in relation to environmental variables,
423 unlike most community analyses that can only directly compare two assemblages at a time.
424 Moreover, SEMs standardise across environmental variables without the need for multiple

425 tests that risk false positives, and can, therefore, elucidate the relative strengths of
426 explanatory variables in driving observed relationships.
427
428 *Applications*
429 An effective surrogate should be transferable over a broad context and offer a
430 currency that is understandable to a range of stakeholders. According to our findings,
431 macrophytes could meet these criteria in providing an indirect surrogate for molluscs, beetles
432 and dragonflies in ponds and for molluscs in lakes. Macrophyte richness as a freshwater
433 biodiversity surrogate could applicable from local to landscape scales, and simplify complex
434 patterns and processes. By isolating the effects of multiple environmental and spatial
435 explanatory variables in our dataset we demonstrate statistically that, via the diversity of
436 morpho-group diversity, a greater richness of macrophytes is also broadly indicative of
437 greater richness across disparate macroinvertebrate groups in ponds and molluscs in lakes.
438 From an applied perspective, as macrophytes act as ecosystem architects, our findings
439 suggest that researchers or practitioners can straightforwardly obtain a broad indication of the
440 overall habitat quality and macroinvertebrate biodiversity by monitoring the number of
441 macrophyte species and diversity of macrophyte morpho-groups, especially in the case of
442 ponds. Despite the advantages of surrogates, they cannot replace detailed surveys of
443 taxonomic groups particularly where species are rare, specialists or of conservation interest.
444 Therefore, although our results show that macrophyte morpho-group diversity can be useful
445 to indicate freshwater biodiversity, some caution is required as these results may not be
446 definitive in the broad sense.
447 It has been argued that declines in macrophyte richness should be viewed as an early
448 warning system for declines in overall macrophyte abundance and hence the quality of the
449 wider environment (Sayer *et al.*, 2010). Hence, we would recommend practitioners and

conservation managers need to be concerned for wider biodiversity if macrophyte richness begins to decrease. The use of macrophytes as freshwater biodiversity surrogates can be important for rapid and cost-effective assessment of conservation and restoration projects, however, they will be most effective where constraints to biodiversity are diagnosed and addressed at site, habitat and landscape-scales. For example, at the site-scale, high grazing pressures may limit macrophyte regeneration from seedbanks and therefore wider biodiversity will only benefit if areas of macrophytes are protected from over-grazing and high disturbance. Additionally, at the habitat or landscape-scale, species translocations may be needed to enhance structural complexity if there are significant barriers to colonisation. However, in using macrophytes as a proxy for wider biodiversity, particularly when assessing habitat restoration, it should be recognised that macrophyte responses to management are complex and can be highly variable (Phillips, Willby & Moss, 2016).

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Data Availability Statement

All data referred to in this article and code used in analyses are deposited in DataSTORRE - the University of Stirling research data repository.

Conflict of Interest Statement

The authors declare that they have no conflicts of interest in presenting this work for publication.

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687 Table 1. A summary of environmental characteristics per waterbody type and hydroscape;
688 mean ± SE (min-max). Land use is representative from within catchments for lakes and the
689 surrounding 500 m buffer for ponds.

Waterbody type	Hydroscape (No. waterbodies surveyed)	Size (ha.)	Altitude (m)	Urban (%)	Agriculture (%)	Freshwater (%)	Wetland (%)
Lake	Upland (n=27)	103.7 ± 57.1 (1.5 – 1435.8)	166.9 ± 20.5 (41.0 – 469.0)	0.4 ± 0.2 (0.0 – 2.8)	14.0 ± 4.1 (0.0 – 83.2)	7.0 ± 0.9 (0.8 – 19.4)	0.1 ± 0.1 (0.0 – 0.3)
	Urban (n=22)	15.3 ± 4.8 (1.4 – 81.9)	93.3 ± 11.6 (23.0 – 217.0)	17.1 ± 5.1 (0.0 - 90.8)	33.2 ± 4.1 (0.0 – 69.3)	7.5 ± 1.3 (0.0 – 19.1)	4.2 ± 1.8 (0.0 – 27.2)
	Agricultural (n=25)	14.5 ± 3.4 (1.0 – 57.6)	14.6 ± 4.5 (0.0 – 78.0)	4.2 ± 1.0 (0.0 – 16.9)	61.7 ± 5.1 (2.0 – 88.3)	7.9 ± 2.4 (0.0 – 40.6)	4.9 ± 2.8 (0.0 – 56.0)
Pond	Upland (n=27)	0.4 ± 0.1 (0.1 - 1.6)	160.4 ± 12.3 (64.0 – 306.0)	0.3 ± 0.1 (0.0 – 2.2)	22.2 ± 4.7 (0.0 – 75.5)	1.4 ± 0.5 (0.0 – 12.2)	0.4 ± 0.2 (0.0 – 5.5)
	Urban (n=26)	0.3 ± 0.1 (0.1 - 1.2)	92.5 ± 12.3 (9.0 – 233.0)	39 ± 5.3 (0.0 – 98.9)	33.1 ± 4.9 (0.0 – 94.6)	0.5 ± 0.4 (0.0 – 12.2)	1.1 ± 0.6 (0.0 – 16.6)
	Agricultural (n=30)	0.2 ± 0.1 (0.1 - 1.2)	49.2 ± 5.1 (0.0 – 82.0)	2 ± 0.5 (0.0 – 13.4)	78.3 ± 4.4 (14.4 – 99.4)	0.4 ± 0.2 (0.0 – 4.1)	6.7 ± 2.9 (0.0 – 58.5)

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697 Table 2. Summary of species richness and sampling efficiency per waterbody type and
698 hydroscape for each species assemblage. The estimated sample coverage gives an indication
699 of the sampling completeness of each species group per waterbody type per hydroscape.

Waterbody type	Hydroscape (No. waterbodies surveyed)	Species group	Mean richness (range)	Total richness	Estimated sample coverage (%)
Lake	Upland (n=27)	Macrophytes	20 (11-34)	88	95
		Molluscs	4 (0-22)	22	80
		Beetles	13 (3-30)	86	90
		Odonates	6 (2-13)	19	98
	Urban (n=22)	Macrophytes	25 (12-39)	113	95
		Molluscs	8 (1-15)	28	97
		Beetles	16 (6-26)	68	95
		Odonates	5 (1-10)	10	100
	Agricultural (n=25)	Macrophytes	17 (3-29)	87	94
		Molluscs	16 (3-29)	46	99
		Beetles	20 (5-76)	157	87
		Odonates	16 (5-23)	34	98
Pond	Upland (n=27)	Macrophytes	15 (1-25)	86	95
		Molluscs	2 (0-5)	12	90
		Beetles	15 (3-35)	88	94
		Odonates	10 (6-16)	21	99
	Urban (n=26)	Macrophytes	12 (2-19)	84	90
		Molluscs	4 (0-16)	26	90
		Beetles	11 (2-30)	69	95
		Odonates	5 (1-9)	10	100
	Agricultural (n=29)	Macrophytes	11 (1-26)	95	89
		Molluscs	3 (0-12)	29	95
		Beetles	17 (3-50)	130	90

		Odonates	11 (1-25)	29	99

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Figures

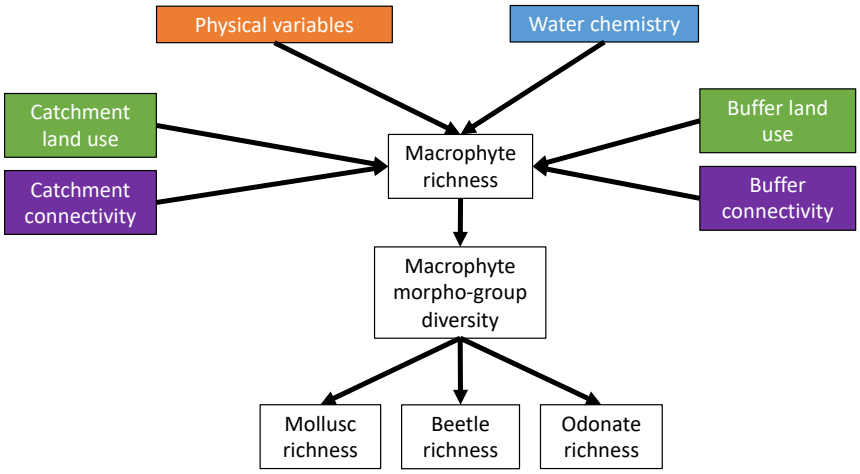

Figure 1. The conceptual model used to illustrate the direct and indirect relationships between response variables (macrophyte richness, macrophyte morpho-group diversity, mollusc, beetle and odonate richness) and explanatory variables (land use, connectivity, physical and water chemistry metrics).

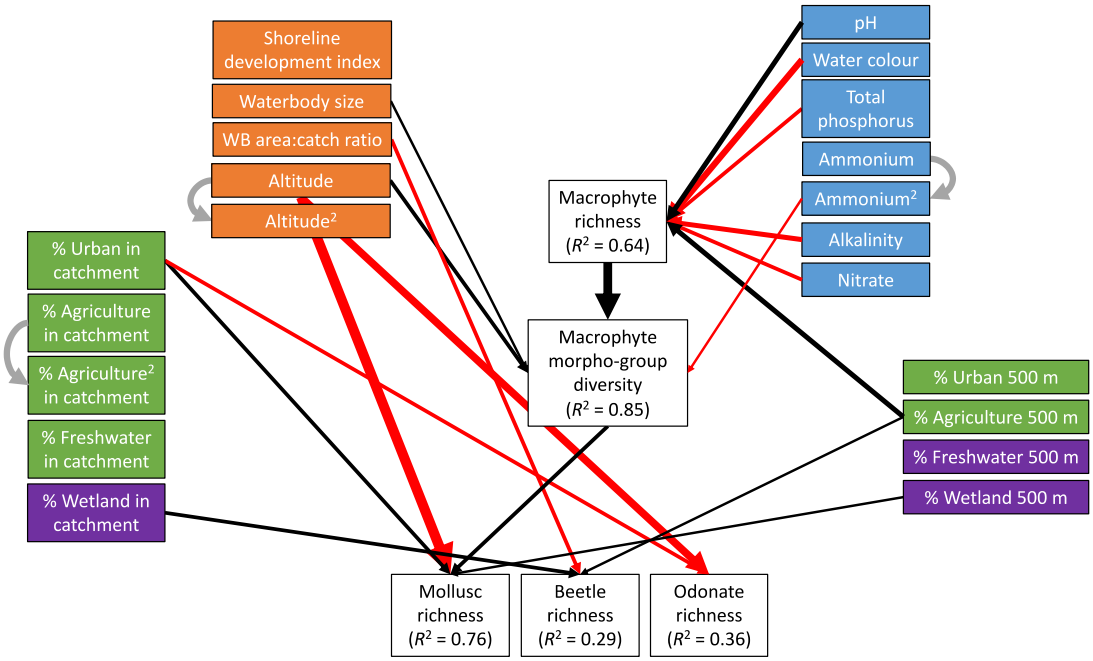

Fig. 2 Structural equation model (SEM) path diagram for lakes. Arrows are scaled according to standardised effect sizes, with black arrows indicating positive effects, red arrows negative

712 and grey arrows indicating specified correlated errors. Explanatory variables with no arrows
713 indicate that they were included in the final SEM but were not significant. Boxes with a
714 superscript represent parameters that had a non-linear relationship with the predictor.
715 Coefficients of determination (R^2) are shown for each response variable. Non-significant
716 relationships (P > 0.05) are omitted for clarity.
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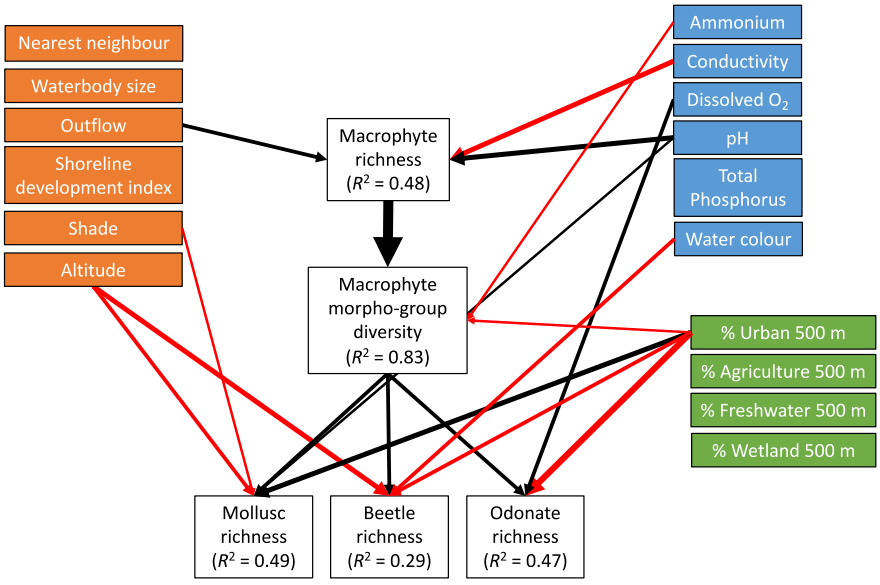

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719 Fig. 3 Structural equation model (SEM) path diagram for ponds. Arrows are scaled according
720 to standardised effect sizes, with black arrows indicating positive effects and red arrows
721 negative. Explanatory variables with no arrows indicate that they were included in the final
722 SEM but were not significant. Coefficients of determination (R^2) are shown for each response
723 variable. Non-significant relationships (P > 0.05) are omitted for clarity.
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